NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 431

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF BENZYL ACETATE

(CAS NO. 140-11-4)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge while supplies last from Central Data Management, NIEHS, P.O. Box 12233, MD A0-01, Research Triangle Park, NC 27709 (919-541-1371).

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

BENZYL ACETATE

(CAS NO. 140-11-4)

IN F344/N RATS AND $B6C3F_1$ MICE

(FEED STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

September 1993

NTP TR 431

NIH Publication No. 93-3162

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

CONTRIBUTORS

National Toxicology Program
Evaluated and interpreted results and reported findings

K.M. Abdo, Ph.D.
C.J. Alden, Ph.D.
G.A. Boorman, D.V.M., Ph.D.
D.A. Bridge, B.S.
S.L. Eustis, D.V.M., Ph.D.
T.J. Goehl, Ph.D.
R.A. Griesemer, D.V.M., Ph.D.
J.R. Hailey, D.V.M.
J.K. Haseman, Ph.D.
G.N. Rao, D.V.M., Ph.D.
C.C. Shackelford, D.V.M., M.S., Ph.D.
B.A. Schwetz, D.V.M., Ph.D.
D.B. Walters, Ph.D.
K.L. Witt, M.S., Oak Ridge Associated Universities

Southern Research Institute
Conducted studies, evaluated pathology findings

J.D. Prejean, Ph.D., Principal Investigator
D.R. Farnell, D.V.M., Ph.D.
H.D. Giles, D.V.M., Ph.D.
J.S. Heath, D.V.M.

Experimental Pathology Laboratories, Inc.
Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
S. Botts, M.S., D.V.M.
B.F. Hamilton, D.V.M., Ph.D.

Dynamac Corporation
Prepared quality assurance audits

S. Brecher, Ph.D., Principal Investigator

NTP Pathology Working Group
Evaluated slides, prepared pathology report on rats (29 May 1991)

T.M. Monticello, D.V.M., Ph.D., Chair
Pathology Associates, Inc.
M.A. Bryant, D.V.M.
U.S. Army Medical Reserves Instructor
B.F. Hamilton, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
M.P. Jokinen, D.V.M.
National Toxicology Program
M.M. McDonald, D.V.M., Ph.D.
National Toxicology Program
S. Qureshi, B.V.Sc., Ph.D.
Sandoz, Ltd.
C.C. Shackelford, D.V.M., M.S., Ph.D.
National Toxicology Program
A. Steber (observer)
University of Wisconsin

Evaluated slides, prepared pathology report on mice (15 August 1991)

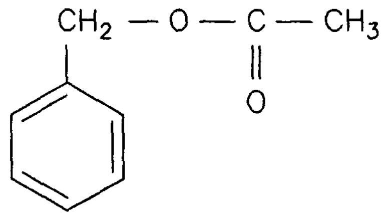
P.K. Hildebrandt, D.V.M., Chair
PATHCO, Inc.
S. Botts, M.S., D.V.M.
Experimental Pathology Laboratories, Inc.
M.P. Jokinen, D.V.M.
National Toxicology Program
J.R. Hailey, D.V.M.
National Toxicology Program
M.M. McDonald, D.V.M., Ph.D.
National Toxicology Program
K.T. Morgan, B.V.Sc., M.R.C.V.S., Ph.D.
Chemical Industry Institute of Toxicology
E. Sander, M.D. (observer)
Bayer AG
C.C. Shackelford, D.V.M., M.S., Ph.D.
National Toxicology Program

Biotechnical Services, Inc.
Prepared Technical Report

D.D. Lambright, Ph.D., Principal Investigator
G.F. Corley, D.V.M.
P.S. Keightley, B.A.
T.A. King-Hunter, B.S.H.S.
H.S. Lindsay, B.A.

CONTENTS

ABSTRACT



BENZYL ACETATE

CAS No. 140-11-4

Chemical Formula: $C_9H_{10}O_2$ Molecular Weight: 150.17

Synonyms: acetic acid benzyl ester, acetic acid phenyl methyl ester, (acetoxymethyl)benzene, acetoxytoluene, benzyl ethanoate, phenylmethyl acetate

Benzyl acetate is used as a flavoring agent in foods, as a fragrance in soaps and perfumes, as a solvent for cellulose acetate and nitrate, and as a component of printing inks and varnish removers. The NTP previously studied the toxicology and carcinogenicity of this chemical in F344/N rats and B6C3F$_1$ mice using the gavage route of administration and corn oil as a vehicle. Benzyl acetate increased the incidences of pancreatic acinar cell adenomas in male rats and the incidences of hepatocellular adenomas and forestomach neoplasms in male and female mice. Because of the confounding effect of corn oil on the incidences of pancreatic neoplasms and because of controversy over the use of the gavage route of administration, the NTP decided to restudy benzyl acetate using the dosed feed route of administration. In these repeat studies, male and female F344/N rats and B6C3F$_1$ mice received benzyl acetate (at least 98% pure) in feed for 13 weeks and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, L5178Y mouse lymphoma cells, *Drosophila melanogaster*, and mouse bone marrow and peripheral blood cells.

13-Week Study in Rats

Groups of 10 male and 10 female F344/N rats were fed diets containing 0, 3,130, 6,250, 12,500, 25,000, or 50,000 ppm (0, 230, 460, 900, 1,750, or 3,900 mg/kg body weight for males and 0, 240, 480, 930, 1,870, or 4,500 mg/kg for females) benzyl acetate for 13 weeks. Nine male and nine female rats receiving 50,000 ppm benzyl acetate died or were killed moribund between weeks 2 and 8 of the study. The mean body weight gain and the final mean body weight of 25,000 ppm males were significantly lower ($P \leq 0.01$) than those of the control group. Feed consumption by exposed rats, except the 25,000 and 50,000 ppm males and 50,000 ppm females, was similar to that by the controls. The reduced feed consumption by 25,000 and 50,000 ppm males and 50,000 ppm females may have been due to toxicity or decreased palatability. Tremors and ataxia occurred only in the 50,000 ppm rats. These findings were first observed on day 15 in nine males and six females and continued until the end of the study. Cholesterol levels in 12,500 and 25,000 ppm females and triglyceride levels in 25,000 ppm females were lower than those in the controls.

Chemical-related lesions occurred in the brain, kidney, tongue, and skeletal muscles of the thigh. Necrosis of the brain involving the cerebellum and/or hippocampus, degeneration and regeneration of the renal tubule epithelium, and degeneration and sarcolemma nuclear hyperplasia of the tongue and skeletal muscles occurred in most male and female

50,000 ppm rats. This effect was observed in the 1,000 mg/kg group in the previous gavage study (NTP, 1986).

13-WEEK STUDY IN MICE

Groups of 10 male and 10 female B6C3F$_1$ mice were fed diets containing 0, 3,130, 6,250, 12,500, 25,000, or 50,000 ppm (0, 425, 1,000, 2,000, 3,700, or 7,900 mg/kg body weight for males and 0, 650, 1,280, 2,980, 4,300, or 9,400 mg/kg for females) benzyl acetate. One 50,000 ppm male mouse died and one 50,000 ppm female mouse was killed moribund before the end of the study. Mean body weight gains and final mean body weights of all exposed male and female mice were significantly lower than those of the controls and the mean body weight gains decreased with increased exposure level. Feed consumption by 3,130 ppm males and all exposed females was lower than that by the controls. Tremors occurred only in females and were first observed on day 16 in three females receiving 50,000 ppm, day 94 in one female receiving 25,000 ppm, and day 93 in one female receiving 12,500 ppm. The tremors continued until the end of the study.

Necrosis of the brain involving the hippocampus occurred in four 50,000 ppm mice, one male and three females. Hepatocellular necrosis also occurred in the male with brain lesions. On reexamination of the previous 13-week gavage study (NTP, 1986), a similar lesion was seen in the brain of one 1,000 mg/kg female mouse; none were seen in 1,000 mg/kg male mice. The lesion was less severe than that described in the present dosed feed study. The highest dose used in the gavage study was 1,000 mg/kg compared to an estimated high dose of 7,200 mg/kg for the feed study.

2-YEAR STUDY IN RATS

The doses selected for the 2-year feed study of benzyl acetate in F344/N rats were based on lower survival, mean body weights, and feed consumption, and on increased incidences of histopathologic brain lesions in 50,000 ppm male and female rats in the 13-week study. Groups of 60 male and 60 female F344/N rats were fed diets containing 0, 3,000, 6,000, or 12,000 ppm benzyl acetate for 2 years.

Survival, Body Weights, Feed and Compound Consumption, and Clinical Pathology

Survival of exposed rats was similar to that of the controls. The mean body weights of the 12,000 ppm males and exposed females were approximately 5% lower than those of the controls throughout most of the study. The feed consumption by 12,000 ppm males was slightly lower than that by the controls. Dietary levels of 3,000, 6,000, and 12,000 ppm benzyl acetate were estimated to result in average daily consumption levels of 130, 260, and 510 mg/kg body weight (males) and 145, 290, and 575 mg/kg (females). No biologically significant changes in hematology or clinical chemistry parameters were found that could be attributed to benzyl acetate administration.

Pathology Findings

No compound-related increased incidences of neoplasms or nonneoplastic lesions occurred in male or female F344/N rats receiving benzyl acetate for as long as 2 years.

2-YEAR STUDY IN MICE

The doses selected for the 2-year feed study of benzyl acetate in B6C3F$_1$ mice were based primarily on lower body weight gains and lower final mean body weights of exposed mice in the 13-week study. Groups of 60 male and 60 female B6C3F$_1$ mice were fed diets containing 0, 330, 1,000, or 3,000 ppm benzyl acetate for 2 years.

Survival, Body Weights, Feed and Compound Consumption, and Clinical Pathology

Survival of all exposed mice, except the 3,000 ppm females, was similar to that of the control groups. Survival of 3,000 ppm females was significantly higher than that of the control group. Throughout the 2-year study, the mean body weights of 1,000 and 3,000 ppm males and females were 2% to 14% lower than those of the control groups. Dietary levels of 330, 1,000, and 3,000 ppm benzyl acetate were estimated to result in average daily consumption levels of 35, 110, and 345 mg/kg (males) and 40, 130, and 375 mg/kg (females). No biologically significant changes in hematology or clinical chemistry parameters were observed in mice receiving 330, 1,000, or 3,000 ppm benzyl acetate.

Pathology Findings

No increase in neoplasm incidence in mice could be attributed to benzyl acetate administration in feed. This contrasts with the previous finding that administration of benzyl acetate in corn oil by gavage once daily 5 days a week for as long as 2 years was carcinogenic to mice, causing increased incidences of hepatocellular neoplasms and forestomach neoplasms. The contrast in results between the two studies may be due to differences in the dose levels used (highest dose: gavage, 1,000 mg/kg a day; feed, 360 mg/kg a day).

Dose-related increased incidences or severities of nonneoplastic nasal lesions occurred in the most posterior portions of the nasal cavity in all exposed groups. The lesions occurred in the majority of the exposed mice and consisted of atrophy and degeneration, primarily of the olfactory epithelium, cystic hyperplasia of the nasal submucosal glands, pigmentation of the mucosal epithelium, and exudate accumulation.

GENETIC TOXICOLOGY

Benzyl acetate was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537, with or without exogenous metabolic activation (S9). However, a positive response was observed for benzyl acetate, with and without S9, in the mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y cells. No significant increases in the frequencies of sister chromatid exchanges or chromosomal aberrations occurred in cultured Chinese hamster ovary cells treated with benzyl acetate *in vitro*, with or without S9, and no increases in either sister chromatid exchanges or chromosomal aberrations occurred in bone marrow cells of male mice treated *in vivo* by intraperitoneal injection. No increase in sex-linked recessive lethal germ cell mutations occurred in male *Drosophila melanogaster* administered benzyl acetate in feed or by injection. Tests of benzyl acetate for induction of micronucleated erythrocytes in bone marrow and peripheral blood of mice were also negative.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** of benzyl acetate in male or female F344/N rats receiving 3,000, 6,000, or 12,000 ppm; however, rats may have tolerated higher doses. There was *no evidence of carcinogenic activity* of benzyl acetate in male or female B6C3F$_1$ mice receiving 330, 1,000, or 3,000 ppm.

Nasal lesions associated with benzyl acetate exposure in male and female mice included nasal mucosa atrophy and degeneration (primarily of the olfactory epithelium), cystic hyperplasia of the nasal submucosal gland, and luminal exudate and pigmentation of the nasal mucosal epithelium.

In previous 2-year gavage studies, benzyl acetate increased the incidence of acinar cell adenomas of the exocrine pancreas in male F344/N rats; the gavage vehicle may have been a contributing factor. There was no evidence of carcinogenic activity in female F344/N rats receiving 250 or 500 mg/kg a day. There was some evidence of carcinogenic activity in male and female B6C3F$_1$ mice, indicated by the increased incidences of hepatocellular adenomas and squamous cell neoplasms of the forestomach.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Benzyl Acetate

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses			
0, 3,000, 6,000, or 12,000 ppm in feed (approximately 130, 260, or 510 mg/kg)	0, 3,000, 6,000, or 12,000 ppm in feed (approximately 145, 290, or 575 mg/kg)	0, 330, 1,000, or 3,000 ppm in feed (approximately 35, 110, or 345 mg/kg)	0, 330, 1,000, or 3,000 ppm in feed (approximately 40, 130, or 375 mg/kg)
Body weights			
12,000 ppm group slightly lower than control	Exposed groups slightly lower than control	1,000 and 3,000 ppm groups lower than control	1,000 and 3,000 ppm groups lower than control
2-Year survival rates			
27/50, 34/50, 30/50, 33/50	30/50, 30/50, 37/50, 28/50	39/50, 43/50, 41/50, 39/50	29/50, 28/50, 37/50, 44/50
Nonneoplastic effects			
None	None	Nose: mucosa atrophy (30/50, 49/50, 50/50, 50/50); mucosa degeneration (31/50, 50/50, 50/50, 50/50); glands, cystic hyperplasia (22/50, 43/50, 47/50, 50/50); exudate (8/50, 18/50, 38/50, 26/50); mucosal pigmentation (0/50, 45/50, 50/50, 50/50)	Nose: mucosa atrophy (41/50, 48/50, 49/50, 50/50); mucosa degeneration (48/50, 48/50, 50/50, 50/50); glands, cystic hyperplasia (39/50, 45/50, 49/50, 50/50); exudate (15/50, 26/50, 36/50, 43/50) mucosal pigmentation (0/50, 46/50, 48/50, 48/50)
Neoplastic effects			
None	None	None	None
Level of evidence of carcinogenic activity			
No evidence	No evidence	No evidence	No evidence

Genetic toxicology

Salmonella typhimurium gene mutation:	Negative in strains TA98, TA100, TA1535, and TA1537 with or without S9
Mouse lymphoma gene mutations:	Positive with and without S9
Sister chromatid exchanges	
Cultured Chinese hamster ovary cells *in vitro*:	Negative with or without S9
Mouse bone marrow *in vivo*:	Negative
Chromosomal aberrations	
Cultured Chinese hamster ovary cells *in vitro*:	Negative with or without S9
Mouse bone marrow *in vivo*:	Negative
Sex-linked recessive lethal mutations	
Drosophila melanogaster:	Negative when administered in feed or by injection
Micronuclei induction	
Mouse bone marrow *in vivo*:	Negative
Mouse peripheral blood *in vivo*:	Negative when administered in feed for 13 weeks

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on benzyl acetate on December 1, 1992, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On December 1, 1992, the draft Technical Report on the toxicology and carcinogenesis studies of benzyl acetate received public review by the National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. K.M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of benzyl acetate by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related nonneoplastic lesions in male and female B6C3F$_1$ mice. Benzyl acetate was studied previously by the NTP using the gavage route with corn oil as the vehicle. Because of the confounding effect of corn oil on the increased incidence of pancreatic neoplasms in male rats, the NTP decided to restudy the chemical using the dosed feed route. Dr. J. Yuan, NIEHS, reported on pharmacokinetic studies designed to compare the internal dose for the feed route with that for the gavage route. The pharmacokinetic studies demonstrated that blood levels of the major metabolite of benzyl acetate, benzoic acid, were up to 300 times greater after gavage administration than after administration in the feed. The proposed conclusions for the current and previous studies were *no evidence of carcinogenic activity* of benzyl acetate in male or female F344/N rats, and *no evidence of carcinogenic activity* of benzyl acetate in male or female B6C3F$_1$ mice.

Dr. Davis, a principal reviewer, agreed in principle with the proposed conclusions. He thought a maximum tolerated dose (MTD) was not achieved in rats. Based on lack of mortality or clinical signs and only a modest effect on weight gain at 25,000 ppm in the 13-week studies, he suggested that the highest dose in the 2-year studies should have been between 25,000 and 50,000 ppm. Dr. Abdo agreed that rats could have tolerated a higher dose, but during dose selection there was concern about reduced feed consumption. Dr. Davis requested that this information be added to the report.

Dr. Carlson, the second principal reviewer, agreed with the proposed conclusions, and also felt that the MTD was not reached in the rat studies. He asked whether it was true that nasal lesions did not occur in previous studies or whether they were just not looked for. Dr. C.C. Shackelford, NIEHS, said there was no evidence of nasal lesions in the previous NTP study.

Dr. van Zwieten asked whether the rationale for repeating this as a feed study included reasons other than the corn oil effects noted in the first study. Dr. Abdo cited forestomach irritation as a reason, as well as the fact that human exposure to benzyl acetate is more often through dermal contact or by ingestion of contaminated food. Dr. Davis asked for staff comment on the importance of reaching the MTD. Dr. G.A. Boorman, NIEHS, said it was quite important, particularly when trying to compare effects in studies involving different routes of chemical administration such as this. Dr. Klaassen criticized the analytical method used for measuring blood levels of benzoic acid and also wondered whether the toxicology of benzoic acid was being studied instead of benzyl acetate. Dr. T.J. Goehl, NIEHS, said the method was sensitive down to 1 μg/mL of benzoic acid and can also detect benzyl alcohol and hippuric acid; however, benzoic acid is the major component after either gavage or feed administration. Dr. B.A. Schwetz, NIEHS, said an important point in giving perspective to both studies is that the metabolism of benzyl acetate in rodents is probably quite similar to that in humans.

Dr. Davis moved that the Technical Report on benzyl acetate be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, *no evidence of carcinogenic activity*. Dr. Carlson seconded the motion and then offered an amendment that the statement be added to the conclusions that higher doses could have been tolerated in the 2-year rat studies. Mr. Beliczky seconded the amendment, which was accepted by six yes votes (Mr. Beliczky, and Drs. Brown, Carlson, Davis, Taylor, and Ward) to four no votes (Drs. Bailey, Davidson, Ryan, and van Zwieten). The original motion by Dr. Davis as amended by Dr. Carlson was then accepted unanimously with ten votes.